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VIRAL GASTROENTERITIS

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16. ABSTRACT

Two virus types have been clearly shown to have epidemiologic importance in viral gastroenteritis, i.e., rotavirus and Norwalk virus. Four other virus types have been associated with gastroenteritis but their epidemiologic importance is not yet known, i.e., enteric adenovirus, calicivirus, astrovirus and a goup of small round viruses that are indistinct morphologically and are Norwalk-like. In addition, there are a few other unculturable viruses found in human stools that are suspected of causing gastrointestinal illness, i.e., enteric coronavirus-like viruses and other small round viruses such as mini-rotavirus and mini-reovirus. This report reviews the current information on the biological characteristics, clinical features, epidemiology and other aspects of these viral agents.

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BACKGROUND

Acute infectious diarrhea is a major medical problem in developing nations where it causes considerable morbidity and mortality. This syndrome also accounts for substantial misery and loss of work time in developed countries even though mortality rates are low. A large proportion of acute infectious diarrhea is not cause by recognizable bacteria or parasites. It has only been during the past decade that viral agents have been discovered and shown for the first time to be medically important causes of infectious diarrhea. Earlier efforts to discover and implicate viral agents in infectious diarrhea repeatedly failed, because laboratory techniques were confined to the use of standard tissue cultures and conventional laboratory animals. It was only when new laboratory approaches were employed that viruses producing gastroenteritis were at last discovered. These new laboratory methods included foremost the use of electron microscopy to examine clinical specimens, together with immunoassays to detect viral antigens in stools, and the use of unusual culture techniques to uncover complete or partial replication of viruses.

During the 1960's it was suspected that described viruses known to replicate in the gastrointestinal tract such as readily cultivatable adenoviruses, echoviruses and coxsackieviruses, were causes of viral gastroenteritis. Careful epidemiological studies, however, revealed that these viruses were found in similar frequencies among children with and without diarrhea. Thus, these agents were not the long elusive causes of viral gastroenteritis. The echo and coxsackie viruses are enteric only in the sense that they enter the host via

the gastrointestinal tract, multiply in the intestine, and then produce viremia with their eventual pathogenesis occurring in tissues such as skin (rash), central nervous system (meningitis), or heart (myopericarditis).

Epidemiologic and Clinical Patterns

Viral gastroenteritis syndromes are second in frequency only to the common cold among illnesses affecting American families under epidemiological surveillance (1). These syndromes fit mostly into two medically important epidemiologic patterns. The first is typically epidemic and produces family and community outbreaks of illness among schoolchildren and adults. This pattern characteristically consists of a 24 to 48 hour self-limited illness with a sudden, explosive onset of varying combinations of diarrhea, vomiting, abdominal cramps, nausea, headache, malaise, myalgia, anorexia and low-grade fever. Norwalk virus is a common cause of this form of illness.

The second pattern is commonly sporadic, occasionally epidemic, and usually occurs in infants and young children. Illness typically consists of 5 to 8 days of severe diarrhea, usually accompanied by vomiting and fever. Severe dehydration may develop in ill infants, necessitating fluid replacement. This form of illness is common during cooler months in temperate climates. Older children and adult contacts of ill infants can develop overt illness but infection is usually mild or asymptomatic. Rotavirus is a common cause of this pattern of illness.

Pathogenesis

The pathogenesis of viral gastroenteritis has been best studied in volunteers experiencing experimentally induced illness, particularly that due to Norwalk virus. Data also exist from naturally occuring illness due to other viral gastroenteritis agents, principally rotavirus. Viral gastroenteritis is an infection of the small intestine, and usually does

not involve the large bowel. During illness, a histologic lesion of the mucosa of the proximal small intestine is found, which typically reverts to normal within two weeks (2). This transient lesion is accompanied by small intestinal dysfunction which is reflected in the malabsorption of d-xylose. Histologically, the gastric and colonic mucosa remain normal. Hence, during illness, blood and leukocytes are rarely seen in stools. This finding represents a helpful clinical diagnostic clue, inasmuch as a viral cause for gastroenteritis should not be entertained if a patient's stool contains fecal leukocytes. In strictly histologic terms, viral gastroenteritis should be viewed as an "enteritis" since only the small intestine is involved morphologically. The illness is certainly not a "colitis". However, abnormal gastric physiologic emptying is seen in enteric illness of viral etiology, and thus the term viral "gastroenteritis" seems appropriate.

Etiologic Agents

Currently, gastroenteritis viruses can be considered within three categories, as shown in Table 1. The medical and major epidemiologic importance of rotavirus and Norwalk virus are well demonstrated, and much of this chapter will be devoted to a discussion of these two agents. In addition, there are several agents that clearly produce gastroenteritis such as enteric adenovirus, calicivirus, astrovirus, and certain small round viruses; however, the medical importance of these agents has yet to be conclusively shown through definitive epidemiological studies. Currently available data suggest that enteric adenovirus and calicivirus may be medically important agents. Finally, there exist putative agents suspected of producing viral gastroenteritis,such as enteric coronavirus-like particles and certain small round virus-like particles.

The identification and characterization of gastroenteritis viruses, combined with an understanding of their immunology and epidemiology, are necessary in order to prevent their transmission and to develop vaccines where possible. This is a large task because all of the currently recognized and putative agents of viral gastroenteritis share the property of being initially noncultivatable by conventional cell culture or laboratory animal techniques. Nonetheless, striking progress is being achieved in our understanding of viral gastroenteritis to the extent that initial trials with a rotavirus vaccine are underway. This chapter will discuss and place into current perspective each of the recognized and putative agents of viral gastroenteritis, beginning with rotavirus.

ROTAVIRUS

This is the most studied and best understood gastroenteritis virus. Its viral classification and many features of its basic virology, antigenic nature, epidemiology and pathogenesis are now recognized. Although our understanding of the clinical immunology of rotaviral illness is not fully elucidated, the need for disease prevention is clearly recognized and thus vaccine development and trials have been started.

Human rotavirus was first discovered in 1973. It is now recognized as a major pathogen of infants and young children, and is responsible for approximately half of hospitalizations worldwide for gastroenteritis in children under three years of age. The virus is 70nm in diameter, lacks an envelope but has an inner and outer capsid, and possesses eleven segments of double-stranded RNA for its genome. It is now classified as a separate genus of the reoviridae family. The name of the virus is derived from the Latin word "rota", meaning wheel, to which it bears some structural similarity. The human rotavirus is morphologically similar to rotaviruses derived from many other animal species in which they produce neonatal diarrhea. Moreover, human rotavirus shares certain common

antigens with animal rotaviruses, a feature which can be exploited for vaccine development and diagnostic purposes. Although many animal rotaviruses are readily grown in cell culture, special techniques are required for the cultivation of human rotavirus.

The biological characteristics of rotavirus will be discussed first, with emphasis on those features relevant to the consideration of disease prevention. This will be followed by an updated review of clinical, diagnostic and epidemiologic aspects of the virus, and finally by a discussion of treatment and disease prevention.

Biological Characteristics

MOLECULAR BIOLOGY - An understanding of the molecular biology of rotavirus has become important in the planning of strategies for disease prevention and control. This is due to the current recognition of several serotypes of rotavirus with a possible lack of complete cross-protection among serotypes. Furthermore, genetic recombinant rotaviruses may occur in nature. The functions of different rotaviral genes are now being understood, and particular interest has been directed towards recognition of the role that each rotaviral gene plays in determining viral growth, infectivity, and induction of host immunity.

The eleven double-stranded RNA genes of animal and human rotaviruses and their protein translation products have been analyzed by a variety of biochemical, gene cloning, genetic and immunologic techniques. Analyses have revealed important virologic functions associated with certain genes. For example, gene 9 (in some strains gene 8)codes for an outer capsid glycoprotein which has been identified as the major virus neutralization antigen (3-8). It is responsible for serotype specificity of rotavirus, and is likely important in protective immunity (9,10). A lesser virus neutralization function is associated with gene 4 and its coded product of another outer capsid protein. This protein is responsible

for the restriction of viral growth in cell culture, and also serves as the viral hemagglutinin (7,11,12). In contrast, gene 6 codes for an inner capsid protein responsible for a common antigen shared by many animal rotaviruses, and also confers subgroup characteristics of rotaviruses (see "CLASSIFICATION" below)(13). Thus, molecular genetic analysis of rotavirus has already revealed specific genetic factors responsible for serotype-specific neutralization of the virus, restriction of viral growth in cell culture, and common group-specific reactivity shared by different rotaviruses.

CLASSIFICATION - It is only recently that the serotypic classification of rotavirus has been clarified. Previously, the word "serotype" was used in the literature to define rotaviral strains that differed when analyzed by any of several immunological techniques (14,15). It is now known, however, that strains which differ when studied by enzyme-immunoassay, complement-fixation, counterimmunoelectrophoresis, and immune adherence hemagglutination do not represent distinct serotypes; rather they differ on the basis of their reactivity with the major inner capsid protein, the product of gene 6 (7,16). Earlier literature defined these strains as "serotypes", whereas today they are designated by the term subgroup. This confusion is understandable, in retrospect, because human rotavirus was not cultivated in vitro until 1981, and prior to that time antigenic analysis needed to rely primarily on the immunological techniques mentioned above. However, with the in vitro cultivation of the virus, strains were then able to be defined by neutralization of their infectivity in cell culture. Thus, the term serotype is now used to identify the antigen that reacts with neutralizing antibodies as is customary for most viruses. Serotype specificity is determined by an outer capsid glycoprotein coded for by gene 8 or 9, and is likely related

to protective immunity (9,10).

Table 2 summarizes the classification of rotavirus. At present, at least four human rotavirus serotypes have been defined (9,17-20). In addition, two, and possibly three, subgroups are described (13-15,21). It is interesting that human and some animal rotaviruses are reported to be in the same realm of classification for both serotype and subgroup. Most mammalian animal rotaviruses are of subgroup 1, of which two simian strains and a canine rotavirus also belong to serotype 3, thus bearing a very close immunological relationship with one human rotavirus serotype (22).

In addition to the classification by serotype and subgroup, rotavirus has also been classified by analysis of the mobility of viral RNA segments during polyacrylamide gel electrophoresis (23,24). This methodology circumvented cultivation of the virus and the use of standardized typing serum reagents, and it also enabled an evaluation of all rotaviral genes rather than just a few selected antigens. However, in time it has become apparent that there are numerous strains definable by electropherotype classification with variability of strains in large outbreaks (25-32). In addition, there are complexities involved in this approach. For example, different serotypes have been shown to have identical electropherotypes, and rotaviruses with different RNA homology by hybridization techniques have also been shown to have identical electropherotypes (33,34). It is therefore clear that comigration of equivalent segments of different rotaviruses does not guarantee their identity nor does the lack of comigration always indicate significant variation. Electropherotypic study of rotaviruses is, however, of some value in epidemiological monitoring of the spread of virus from one individual to another in a limited outbreak, and in helping to study the evolution of new rotavirus strains through antigenic shift and drift (30,31).

IN VITRO CULTIVATION - Human rotavirus in clinical fecal specimens was cultivated in cell culture using a combination of techniques that, individually, had been previously unsuccessful (9,35-38). These procedures included the use of roller cell cultures, the incorporation of trypsin into the culture medium, and the treatment of the viral sample with trypsin. Representatives of all serotypes and subgroups have been cultivated. However, in vitro cultivation is at present not a viable diagnostic method for rotavirus because of relatively low isolation rates and the frequent need for numerous initial blind passages. Immunoassay techniques (discussed below under "Diagnostic Tests") are much more sensitive for the recognition of rotavirus in stool specimens, and can be performed quickly. At the present time, in vitro cultivation of rotavirus is an invaluable research tool for study of the virus and may eventually be needed for vaccine production.

Clinical Features

Symptomatic illness is most common in children between 6 months and 2 years of age (39,40). Neonates may become infected, but usually they are asymptomatic even though they shed the virus (41-43). Adults characteristically experience asymptomatic infection or mild symptoms (40, 44-46); however, severe disease can occasionally occur in adults (47,48).

The incubation period for infection is 1 to 3 days (49,50). Illness typically begins with watery diarrhea, vomiting and low grade fever. Vomiting is more commonly seen in rotavirus diarrhea than in other forms of pediatric gastroenteritis (51). The mean duration of rotavirus illness is 5 to 8 days (52). The virus is commonly detected in stools for 9 days after the onset of illness (53,54). Disease is usually self-limited in developed areas, although occasionally symptomatic relapses can occur (55,56). When fatalities occur, they are usually found in infants and young children residing in developing areas of the world; death has also

been reported in elderly patients with severe underlying nondiarrheal disease (48,57).

Isotonic dehydration and compensated metabolic acidosis are common clinical features. The level of dehydration is typically less than 5 percent, but increases to 10 to 20 percent in many fatal cases (51,55, 57,58). In rotavirus diarrhea, there is an intestinal malabsorption of salt and water, resulting in a net fluid secretion with increased concentrations of sodium and chloride in stools (56,59). Feces are usually liquid, lacking blood and leukocytes; mucus is sometimes seen (51,58). Pale, fatty stools have been noted in some cases, suggesting impairment of both the digestion of fat and pigmentation of feces (52,60).

Upper respiratory tract symptoms have been noted occasionally in children with rotavirus gastroenteritis in whom no evidence has been found for viral or bacterial respiratory pathogens (51,61,62). However, rotavirus has not been detected in the respiratory tract of patients with gastroenteritis (50,62). Nonetheless, the rapid person to person spread of rotavirus infection may be more consistent with a respiratory than just a fecal-oral route of transmission (63-65). One report has detected rotavirus in the respiratory tract secretions of 4 patients with pediatric pneumonia, 3 of whom lacked diarrhea (66). Confirmation is needed of this observation, together with a definitive assessment whether or not rotavirus can cause pneumonia or merely colonize the respiratory tract.

There are reports, all requiring confirmation, potentially linking rotavirus infection with intussusception, Reye's syndrome, encephalitis, sudden infant death syndrome, inflammatory bowel disease, and neonatal necrotizing enterocolitis (67-72). At present, there is no persuasive evidence to suuport any of these associations etiologically.

Diagnostic Tests

The method by which the specific diagnosis of rotavirus infection is made differs from that usually used for most other viral infections. Rotavirus infection can be diagnosed quickly by the direct detection of the virus or its antigens in stool samples. This is in contrast to many other viral infections which typically are recognized by the isolation over days to weeks of the virus in cell cultures or laboratory animals, or by the demonstration retrospectively of a seroconversion to the virus. Although rotavirus can now be grown in cell culture from some clinical stool samples (9, 35-38), the virus is isolated from only 50 to 70 percent of stool specimens known to contain the virus by electron microscopy or immunoassay. Thus, cultivation is not a practical diagnostic technique for rotavirus.

Electron microscopy is the standard by which other diagnostic assays for rotavirus must be judged. It provided the methodology for the original identification of the virus in stools and is a sensitive and reliable technique. However, its use is limited by the requirement for specialized equipment and personnel as well as by difficulties in handling large numbers of specimens.

At present, the diagnostic method of choice for most situations is solid-phase enzyme-linked immunoassay (73-75). It detects rotavirus in stool samples within 4 to 24 hours and can be performed on large numbers of specimens with routinely available equipment and personnel. The principle of this type of assay is as follows: a test stool sample is exposed to a solid phase which has already been coated with antibody to rotavirus. Then, in a "direct" test, an enzyme labeled virus-specific detection antibody is applied to demonstrate rotavirus

bound to the solid phase. In an "indirect" test, an unlabeled anti-rotavirus serum (prepared in a second animal species different from the origin of the coating serum) is added followed by an enzyme labeled antiglobulin directed against the second animal species.

Initial serious problems with nonspecific reactions encountered by immunoassays for rotavirus have now been overcome. Some early literature (76) using rotavirus immunoassay, however, must now be reevaluated in light of new information. One comprehensive study has demonstrated that 73 percent of 1834 fecal specimens reported to be positive by an early described "indirect" test were in fact falsely positive (77). The technical reasons for these nonspecific reactions are not fully understood; however, specific measures to avoid them are now employed successfully in many rotavirus immunoassays. In principle, these measures involve confirmatory and antibody blocking procedures (74,77).

The commercial Rotazyme (Abbott Laboratories, North Chicago) immunoassay does not have a confirmatory test built into its procedure. However, Rotazyme has not encountered major difficulties with false positive results, apparently because of the test's "direct" design and special formulations of sample diluent and wash solutions (78-80). One report, requiring confirmation, does indicate that Rotazyme has encountered frequent false positive reactions in stool specimens from neonates (81). Rotazyme which is based on the use of anti-simian rotavirus serum (82) seems to be somewhat less sensitive in detecting rotavirus than most electron microscopic or "indirect" immunoassays. In practical terms, this relative insensitivity is not noted with the diagnosis of pediatric illness in which large amounts of virus are shed, but it is encountered in adult rotavirus diarrhea in which smaller amounts of virus are present. Recent data indicate that the replacement of polyclonal coating antibody with rotavirus-specific monoclonal antibody improves the sensitivity and

specificity of the rotavirus "direct" immunoassay (83).

Other immunologic techniques to detect rotavirus antigens in stool samples have been developed but are mostly less sensitive than solid-phase immunoassay. These include counterimmunoelectrophoresis, complement fixation, immune adherence hemagglutination and other agglutination techniques (74). Direct detection of rotaviral RNA in polyacrylamide gels has also been used (84). A recently described dot hybridization assay for recognition of rotavirus RNA in diarrheal stools appears to be highly sensitive and specific and should be useful for epidemiological studies (85).

Epidemiology

Rotavirus is a ubiquitous agent, and, world-wide, the great majority of adults and children older than 2 years have serum antibodies to the virus (40,86,87). These antibodies are acquired after either symptomatic or asymptomatic infection (50). It is not known how long detectable antibodies persist in the serum following acquisition, and their precise roles in protection are unclear.

Most rotaviral illness occurs in children 6 to 24 months of age (39, 40). The virus is responsible for about half of the hospitalized cases of diarrheal illness in this age group (39,40). In neonates, infection is usually asymptomatic or very mild (41-43), although exceptions have been reported (86). Adults are less likely to become infected than children, and when infected, adults are more frequently asymptomatic than children (40,44-46). Rotavirus infections in adults are often seen among close contacts of young children (40,45,46). However, this is not always the case, as infection has occurred in the absence of any obvious contact with children among military personnel, hospitalized patients, adult travelers, and the institutionalized elderly (48,88-93). Although symptomatic dis-

ease in adults is usually mild, severe symptoms have been reported in geriatric patients and in apparently normal young adults in Thailand (47,48).

The major source of rotavirus in the community is children, with infection usually being spread from person to person by the fecal-oral route (45,94). In temperate climates, illness occurs mostly during winter months (40). These seasonal waves of rotavirus infection have been observed to be more regular and predictable than infections with any other pediatric pathogens studied in the Washington, D.C. area (39). Low indoor relative humidity during winter has been proposed as being important in enhancement of rotavirus transmission, with young infants confined in close quarters indoors (95). In addition, indoor crowding has been suggested to facilitate indirectly an airborne route of transmission which may not be strictly respiratory but due instead to environmental contamination by fecally shed virus (96). Although the seasonality of rotavirus infection in temperate climates is predictable, exceptions have been noted. Finally, common source outbreaks of infection seem to be the exception, not the rule, even though rare community-wide epidemics of rotavirus diarrhea have been associated with fecally contaminated drinking water (97).

Nosocomial spread of rotavirus is well described, and can be a troublesome problem on pediatric wards (98). Transmission of infection may be aided by asymptomatic or mildly ill infected hospital staff. Rotavirus can also spread between patients via contact with the hands of uninfected attendants (99). Handwashing by hospital personnel, cohorting of infected infants, and even closing affected wards are important infection control measures. It should be noted that hospital personnel can acquire symptomatic infection from ill pediatric patients.

Infection with the two major rotavirus subgroups occurs simultaneously

in a population(14,100). In most areas, infections with subgroup 2 have occurred more frequently than with subgroup 1 (14). A small proportion of rotaviruses detected have recently been classified as a third subgroup (21). Clinical symptoms associated with the well studied first two subgroups appear to be similar. The epidemiology of rotavirus serotypes has yet to be studied in detail.

Clinical Immunity

The specific immune mechanisms involved in protection from disease and in recovery from infection are incompletely understood. It is clear that the clinical humoral antibody response to rotaviral infection is heterotypic in nature. For example, post-infection convalescent human sera are not useful in serotyping rotavirus in contrast to hyperimmune animal sera which clearly distinguish between serotypes by neutralization tests (10,101). In a human volunteer study involving 18 adults, serologic responses revealed cross-reactivity between some viral serotypes and subgroups (50). Similar heterotypic and heterosubgroup responses have been noted in infants and young children apparently undergoing primary infection with rotavirus (50). This broad nature of immunologic responses may have relevance to potential vaccine development, whereby, for example, an animal rotavirus or genetically altered human rotavirus could serve as an effective nonpathogenic immunogen (102, 103).

In adult human volunteers, presence of preinoculation serum neutralizing antibody to either serotype 1 or 2 correlated with resistance to illness caused by the serotype 1 challenge virus (50). However, preexisting local neutralizing activity in the intestinal fluid of these volunteers could not be associated with resistance. Following illness, volunteers mounted both homotypic and heterotypic intestinal fluid neutralizing activity responses.

Prior exposure to rotavirus seems to confer some clinical protection against subsequent reexposure (41), although this observation needs to be correlated more thoroughly with analysis of specific infecting rotavirus serotypes. It is known that children rarely experience severe rotavirus gastroenteritis more than once, but sequential infections by different serotypes or subgroups have been observed (14, 41,104). In a prospective longitudinal study of young children, neonates were monitored for the first 2 weeks of life for rotavirus excretion and then kept under serological surveillance for 3 years (41). Symptoms subsequently associated with the first postneonatal rotavirus infection were less frequent and less severe in children who had experienced neonatal infection than in those who did not. Whereas neonatal infection by one strain of rotavirus did not confer immunity against reinfection by other strains, it did provide protection against the development of clinically severe disease during reinfection. In two adult volunteers, rechallenge with the same viral serotype 19 months after an induced illness failed to produce recurrent diarrhea although mild symptoms occurred in one volunteer (50).

In addition to its incompletely defined role in protection, the humoral immune response apparently also plays a role in the recovery from rotavirus infection. For example, chronic, symptomatic infection with viral excretion persisting for more than 6 weeks has been described in two immunodeficient patients, one with X-linked agammaglobulinemia and the other with severe combined immunodeficiency (105). Another study has shown that convalescent serum antibody titers were significantly lower in patients with severe or prolonged rotavirus illness than in subjects with mild or moderate disease (106).

The potential role of breast milk in the modification of rotavirus infection is uncertain. Many breast milk studies are difficult to inter-

pret conclusively because of problems in controlling for sanitary and socioeconomic conditions, poor definition of the amount and duration of breast feeding and the use of supplementation, as well as the retrospective nature of some studies. It is clear that both breast and bottle fed infants develop rotavirus diarrhea (107); however, in some studies breast fed babies excreted rotavirus significantly less frequently and in lower titers than did formula fed infants (43,108). In contrast, one recent longitudinal study failed to observe any protection against rotavirus infection or illness conferred by breast feeding (96). Rotavirus specific secretory IgA can be detected in breast milk and may persist for many months post-partum (109). However, this in vitro observation has been difficult to correlate with in vivo protective efficacy (108,110). In addition, serotype specificity of milk antibody has yet to be studied. It may be that rotavirus infection occurs only when milk neutralizing antibody capacity is exceeded by the amount of viral expsoure, as occurs in animal model systems. This hypothesis could explain the conflicting data reported in humans.

Thus, only an incomplete picture is currently available for an analysis of the important factors involved in clinical immunity against rotavirus. The role of cell mediated immunity in either protection or recovery has not been studied. Nonetheless, sufficient clinical immunological data are considered to exist in order to embark on vaccine development and trials in view of the need for disease prevention.

Treatment

The most important measure in the treatment of rotavirus diarrhea is fluid replacement. Patients with 5 to 10 percent dehydration, who usually are the very young, elderly or debilitated, require fluid replacement. Oral rehydration with a glucose-electrolytes formula, such as that designed

by the WHO, has been shown to be effective therapy for most dehydrated infants and young children, and is a preferred form of therapy (111,112). More severe degrees of dehydration should be treated with parenteral fluid replacement. Avoidance for one or two days of lactose-containing milk or formulas has also been recommended for non-breast fed infants with moderate to severe diarrhea (111). The course of rotavirus infection and disease has been modified in low birth weight babies by treatment orally with human gammaglobulin containing rotavirus antibodies, with a resultant delayed viral excretion and reduction in the duration and titer of virus excreted (113). Diarrhea was shown to be less severe in gammaglobulin treated babies compared to a placebo group, but potential effects of this therapy on the risk of infection could not be assessed.

Infection control measures for rotavirus in hospitals should be undertaken when indicated. These are discussed above under "Epidemiology."

Anti-viral agents effective in man for treatment of rotavirus disease are not available. Some agents are undergoing trials in animals infected with rotavirus.

Vaccines

Indications exist for rotavirus vaccination in both developed and developing countries. In the former, the virus is responsible for approximately half of pediatric hospitalizations for acute infectious diarrhea. In developing countries, an even greater need exists because the annual death toll in these areas attributable to rotavirus is estimated at one million or more (103). Clearly, young children are the obvious candidates for vaccination. However, adults may be potential vaccine recipients in special circumstances such as the institutional-

ized elderly, travelers, medical personnel in pediatric units (to decrease rotavirus transmission), and perhaps pregnant or nursing mothers in order to induce immunity in their offspring.

As indicated above under "Clinical Immunity," the nature of rotavirus protective immunity in man is incompletely understood. For vaccine development, it is desirable to be able to decide what types of immune responses need to be induced and to which viral antigens these immune responses should be directed in order to achieve protection. An understanding of rotaviral gene functions, classification and clinical immunity is important to plan strategies for vaccine development. Several potential approaches towards vaccine development exist (114). First, the use of an animal rotavirus strain can be attempted if it can be shown to be immunogenic and not pathogenic for man. Recently, an attenuated, cell culture adapted bovine rotavirus "vaccine" was administered to adults as well as young children, and found to be safe and to possess some immunogenic properties (103). This live oral virus when given to two year old children did not produce gastrointestinal or constitutional symptoms nor did it result in virus shedding. One dose induced seroconversion to the homologous virus in 88% of children seronegative by enzyme-linked immunosorbent assay and in 68% of children seronegative by viral neutralization assay. This first report is a promising start for the field of rotavirus vaccine development.

A second approach at vaccine development is the use of reassortant viral strains that share genetic material derived from a human and an animal rotavirus, so that characteristics of immunogenicity and lack of pathogenicity are attained (102,114). The segmented RNA genome of rotaviruses makes this type of genetic manipulation possible

and indeed reassortant strains have already been prepared. A third approach is that of adapting a strain to cell culture in the hope of achieving attenuation, as has been done for other viral vaccines. Such a candidate vaccine could then be analyzed for virulence characteristics based on analysis of its genetic composition. Finally, it may be possible to use as a vaccine purified viral protein(s) coded for by rotaviral gene(s) responsible for induction of serotype-specific neutralizing antibody responses.

Pararotavirus

Recently, a new agent, termed pararotavirus has been discovered in stool specimens from 4 individuals residing in Australia, France, and Bulgaria (115-117). This agent is morphologically indistinguishable from conventional rotavirus; however, it is antigenically distinct in its lack of the group-specific common antigen shared by all previously recognized rotaviruses from mammalian and avian hosts Pararotavirus, like rotavirus, contains 11 double stranded RNA segments; unlike rotavirus, these segments yield a unique and characteristic electrophoretic migration pattern. Since pararotavirus lacks the group-specific common antigen, it is not detected in currently used rotavirus immunoassays. A quite preliminary assessment is that pararotavirus infection is rare and probably not a medically important cause of diarrheal illness. However, considerably more study of pararotavirus is needed before this early assessment is validated.

NORWALK VIRUS

This agent was the first virus discovered to be a medically important cause of gastroenteritis. It is derived from an outbreak of epidemic gastroenteritis that occurred in Norwalk,Ohio in 1968 (1). Four years later, the virus was recognized as a 27nm size particle by immune electron microscopic examination of an infectious stool filtrate derived from the 1968 outbreak (118). The infectivity and pathogenicity of Norwalk virus have been demonstrated by its serial passage in adult volunteers. Norwalk virus is the prototype and best studied member of a group of morphologically similar but immunologically mostly unrelated small round viruses which can be visualized in human stool by electron microscopic techniques. These other "Norwalk-like" viruses, such as Hawaii, W-Ditchling, and Snow Mountain, are of uncertain medical importance; it is not known if they are members of 1 or several viral groups. They are discussed in a later section of this chapter.

Progress in understanding the biology, epidemiology, and immunology of Norwalk virus has been slow because, unlike rotavirus, the virus is small in size, difficult to visualize, shed in relatively low titers in feces, and unable to date to be cultivated in any cell or organ culture system or to produce illness in laboratory animals including primates (94). Despite these obstacles, a surprisingly large body of knowledge exists about this virus to the extent that it is clearly recognized as a major human pathogen. Data gained about Norwalk virus have ultimately depended on human experimentation studies and clinical reagents collected from infected volunteers. When a Norwalk virus-containing human fecal filtrate, devoid of bacteria and toxins, is administered orally to adult volunteers, approximately 50 percent of individuals become ill with diarrhea and/or vomiting. Induced illness is indistinguishable from that which occurs naturally, and no prolonged symptoms or long term sequelae develop (119,120).

Biological Characteristics

Norwalk virus is 27nm in diameter and round in shape; it lacks an envelope and has an unclear substructure (118). It can be purified from human feces by gradient ultracentrifugation and has a buoyant density of 1.36 to 1.41 gm/cm^3 (121). Its infectivity for volunteers remains after exposure to heat, acid or ether; hence, it is a very hardy virus (122). These properties of size, density and stability are similar to those of the DNA-containing parvovirus group, and Norwalk virus has been termed parvovirus-like in earlier literature (119). However, one report suggests that Norwalk virus may actually be a calicivirus (an RNA containing group)because the virus concentrated from feces contains a single 66,000 molecular weight protein characteristic of that group (123). Definitive classification of Norwalk virus cannot be made without demonstration of its nucleic acid, which likely awaits laboratory propagation of the agent because it is shed in stool in amounts too small for analysis of its genome (124).

Clinical Features

Illness occurs predominantly in school age and adult populations (1); infants and young children are usually spared from disease in developed areas (125,126). The incubation period for infection is short, usually 1 to 2 days (119). Illness typically begins abruptly with vomiting and/or diarrhea, accompanied by varying combinations of other systemic manifestations such as malaise, myalgia, headache, and low grade fever (119). Symptoms usually last 1 to 2 days and subside abruptly. The virus has been detected in stool for up to 72 hours after the onset of illness (127). Symptomatic relapses have not been described. Fatalities are very rare, occurring only in the very elderly

or debilitated patient.

Volunteers ill with Norwalk virus develop a characteristic though not unique histologic lesion of the proximal small intestine (2). This lesion disappears within 2 weeks, has occurred in all ill subjects examined as well as in a few asymptomatic volunteers who developed a seroconversion to the virus. Unlike rotavirus, Norwalk virus has not been detected by electron microscopy within small intestinal epithelial cells, probably because of its small size and patchy distribution. During illness, malabsorption of d-xylose and fat occur which may persist for one week even though clinical symptoms have disappeared (119). An abnormal delay in gastric emptying occurs during illness, although the gastric and colonic mucosa remain histologically normal (128,129).

Diagnostic Tests

Norwalk virus cannot be visualized with reliability by standard electron microscopy, due in part to its small size and amorphous surface structure. Rather, immune electron microscopy is necessary to identify the agent in stool samples (118). With this technique, clumped virus-human antibody complexes are visualized by electron microscopy, and the degree of clumping can be used to rate the amount of antibody in an unknown serum specimen. This technique sufficed for the original identification of Norwalk virus and limited pilot serologic studies, but it is cumbersome, time consuming, requiring specialized equipment and personnel, and impractical to perform on large numbers of specimens.

A major breakthrough in the study of Norwalk virus occurred with the development of a radioimmunoassay for the detection of the agent in stools and for the measurement of serum antibody to the virus (130,131).

With this assay, it became possible to test large numbers of specimens, and for the first time to determine the epidemiologic importance of Norwalk virus as a pathogen. The radioimmunoassay test procedure is of a "direct" design and relies entirely on carefully defined fecal and serum samples derived from experimentally infected volunteers. Thus, the test is unfortunately limited to only a few research laboratories that possess small amounts of these valuable reagents of human origin. It has not been possible to prepare hyperimmune animal serum reagents usable for commercial diagnostic purposes because Norwalk virus has not been purified sufficiently from human feces.

Epidemiology

Infection with Norwalk virus occurs worldwide as indicated by seroepidemiologic studies (130-135). Serum antibodies are present in about two-thirds of American adults, are uncommon during childhood, but are acquired during adolescence and early adulthood (130,135). These serologic data are consistent with the failure to implicate Norwalk virus as a cause of gastroenteritis in infants and young children in the United States (125,126). In less developed areas of the world, serum antibodies develop during childhood (133,134), and there is early evidence to suggest that some illness occurs in this younger age group in these areas (132).

The Centers for Disease Control studied 74 outbreaks of acute nonbacterial gastroenteritis from 1976 to 1980, and implicated Norwalk virus as the cause in 42 percent based on seroconversions to the virus by radioimmunoassay in a majority of acute and convalescent sera studied (136). An additional one-quarter of these outbreaks were felt to be caused by related "Norwalk-like" virus(es) because seroconversions occurred but only in a minority of paired sera. From these studies, it

seems possible that only a limited number of viral serotypes cause most outbreaks of nonbacterial gastroenteritis.

These outbreaks have occurred in several settings: in recreational camps, on cruise ships, in areas with contaminated drinking or swimming water, in schools and nursing homes, and with the ingestion of raw shellfish or other uncooked foods such as salads or cake frosting handled in an unsanitary manner (136-147). Some outbreaks associated with food or municipal drinking water have involved thousands of people. In addition, 3 to 15 percent of cases of travelers' diarrhea have been associated with Norwalk virus (89,92,148). Although some forms of epidemic nonbacterial gastroenteritis have been described as "winter vomiting disease" (including the original 1968 Norwalk, Ohio epidemic), Norwalk virus outbreaks have occurred at all times of the year with no striking predilection for any one season (136).

Norwalk virus is transmitted by the fecal-oral route, based on evidence obtained from volunteer studies and epidemiologic reports. Respiratory tract symptoms have not been described, and throat washings from ill volunteers were not infectious when administered to a few subjects by the oral route (122). Nonetheless, because of the very rapid secondary spread of infection in outbreaks, it is conceivable that illness may be transmitted by an airborne route via aerosolized virus-containing vomitus (149).

Clinical Immunity

The pattern of clinical immunity seen with Norwalk virus is unusual and differs from that associated with most common viral illnesses (130,150). Our understanding of this clinical immunity is based on mostly on data derived from human volunteers. Approximately one half of unselected subjects inoculated with Norwalk virus develop illness, which typically occurs in the presence of preexisting serum antibody

(130). Following illness, there is a rise in serum antibody levels. This antibody seems to be a marker for infection in susceptible persons but lacks a protective role. Volunteers who resist illness usually have low to absent levels of serum antibody both before and after exposure to Norwalk virus. Local intestinal tract antibody levels appear to follow the same pattern as serum antibody (124,130). Paradoxically, then, the presence of preexisting Norwalk-specific antibody and the ability to generate it constitute risk factors for this illness.

Long term (27 to 42 month) rechallenge studies indicate that previously ill volunteers redevelop illness (150). Disease resistant subjects remain resistant on such rechallenge. Short term resistance to the virus occurs in that most previously ill volunteers remain well on rechallenge 4 to 14 weeks later (119,124,130,150). It is interesting that small familial clusters of susceptibility and resistance to Norwalk virus have been described in a waterborne disease outbreak in which similar degrees of exposure to the virus for different families were shown epidemiologically (151).

The explanation for the pattern of clinical immunity to Norwalk virus is unknown. One possibility is that there is a genetic control of Norwalk resistance or susceptibility, perhaps on the level of intestinal receptor sites. Some inoculated volunteers have been studied for the histocompatibility loci A,B and D but no correlation with resistance or susceptibility to Norwalk illness has been found. A second possible explanation is that repetitive exposures to the virus throughout an individual's childhood are needed to generate eventual immune response together with illness during adulthood. This latter hypothesis implies that resistant adult volunteers are not primed for ill-

ness due to their fewer naturally occurring previous exposures to the virus than susceptible volunteers. The volunteer studies indicate that immunity to Norwalk virus is not long lasting and that repeated bouts of illness throughout life would seem possible.

The virus-specific serum IgM response to Norwalk agent has been studied in volunteers (152). An IgM response occurs in ill individuals and usually not in non-ill subjects challenged with the virus. The peak IgM response occurs about two weeks after illness, but IgM is detectable for up to 21 weeks after infection. On long term rechallenge, volunteers who were previously ill and had produced IgM antibody again became ill and a secondary response, greater than the first, was seen. It thus seems clear that virus-specific IgM is not necessarily indicative of primary infection with Norwalk virus inasmuch as reinfection produces an enhancement of the IgM response.

Treatment

No specific treatment is available for Norwalk virus illness. Therapeutic use of bismuth subsalicylate in volunteers with gastroenteritis caused by Norwalk virus has produced some reduction in abdominal cramps without; however, affecting the rate of virus excretion (153). Severe dehydration in infected elderly or debilitated patients should be managed with hospitalization for parenteral fluid replacement. Fortunately, this complication is uncommon during Norwalk virus epidemics.

Disease Prevention

Many Norwalk virus infections are associated with contaminated water. A useful approach in infection control would be a readily available test sufficiently sensitive to detect the virus in environmental specimens. Unfortunately, the currently available radioimmunoassay is

not sufficiently sensitive for this purpose.

Prospects for vaccine development seem poor at this time because of the unusual complex pattern of clinical immunity to the virus. The use of a vaccine for producing long lasting immunity seems unlikely in light of the absence of long-term immunity in rechallenged volunteers. Perhaps short-term immunity could be produced by vaccination of individuals at high risk of short-term exposure such as travelers. However, no specific technology exists for producing a vaccine against Norwalk virus since the agent has yet to be cultivated in vitro or to be purified extensively from feces.

GASTROENTERITIS PRODUCING VIRUSES OF UNCERTAIN EPIDEMIOLOGICAL IMPORTANCE

There are several enteric viruses that clearly produce gastroenteritis but their medical importance has yet to be conclusively shown through definitive epidemiological studies. These include enteric adenovirus, calicivirus, astrovirus, and certain small round viruses. These agents will now be discussed in terms of their known biological, clinical and diagnostic features. We will begin with enteric adenovirus followed by calicivirus, both of which may be medically important agents based on currently available data.

Enteric Adenovirus

A newly recognized subgroup of adenoviruses, termed enteric or fastidious adenoviruses, is a cause of diarrheal illness in infants and young children. These viruses differ from the well characterized 39 conventional serotypes of adenoviruses which grow easily in standard tissue cultures; many of these conventional serotypes are well established causes of human illnesses such as respiratory tract infections and conjunctivitis. Although the conventional adenoviruses can be cultivated from the feces of

children with diarrhea, careful epidemiological studies have revealed their presence in the stools of similar numbers of control asymptomatic children (154-157). During the 1970's electron microscopy of stools from patients with pediatric diarrhea was first performed on a wide-scale basis subsequent to the discovery of rotavirus. Adenovirus particles were then demonstrable by electron microscopy; yet, many of these agents unexpectedly failed to grow in standard cell cultures (158,159). A potential medical importance of these noncultivatable adenoviruses in diarrheal illness emerged when they were shown to be more frequently associated with pediatric gastroenteritis than were conventional adenoviruses (125,160,161). It was then recognized that the fastidious adenoviruses purified from the stools of young children with acute gastroenteritis comprised distinct types of adenovirus as determined both by antigenic and DNA restriction endonuclease banding techniques (162,163). Using these methods, two new serotypes of adenovirus have been characterized, designated types 40 and 41, also representing two new subgroups, F and G (164-166). At the present time, these represent the enteric adenoviruses.

The enteric adenoviruses are second to rotavirus in frequency of detection by electron microscopy in the stools of infants and young children with viral gastroenteritis in various parts of the world (39,165,167-169). Enteric adenoviruses may also be found in asymptomatic children but less frequently. For example, in a U.S. study, 3.9 percent of children with acute gastroenteritis shed enteric adenovirus compared with 0.6 percent of controls (125). In a Swedish study, 13 percent of ill children demonstrated adenovirus in their stools compared with 1.5 percent of controls; in this study 70 percent of the adenoviruses visualized were later shown to be enteric adenoviruses (165). The majority (71 percent) of children with enteric adenovirus seroconverted by hemagglutination inhibition

testing whereas seroconversion was not seen in patients shedding conventional adenoviruses.

Enteric adenovirus infection has been reported to occur predominantly in infants and young children, with 85 percent of patients being less than 3 years old (165). Diarrhea is the primary manifestation, and lasts an average of 9 days. Fever and vomiting are reported to be mild, with a mean duration of 2 days. Respiratory symptoms were noted in 20 percent of patients in Sweden and even more frequently in the U.S. (169). No seasonality of enteric adenovirus infection was noted in the U.S. or Finland, but two small seasonal peaks in summer and late winter were reported in Sweden (39,165,168).

Solid phase immunoassays have been developed for the specific detection of enteric adenoviruses in stool specimens (163,165). It is interesting that when standard primary human embryonic kidney cell cultures are infected with enteric adenovirus, intracellular adenovirus-specific antigens can be detected by immunofluorescence, reflecting an abortive replication of the virus (159,161,162). Enteric adenoviruses do replicate completely, however, in a specific cell line, Graham 293, which is an adenovirus type 5 transformed human embryonic kidney cell line (170). It has been proposed that enteric adenoviruses should be functionally defined as those adenoviruses that grow in 293 cells but not in untransformed parental human embryonic kidney cells (171).

Calicivirus

This virus is now recognized as a cause of gastroenteritis. Although animal caliciviruses are well described, no human calicivirus has been reported until the recent association with diarrheal disease (172,173). The human calicivirus is classified on the basis of its

characteristic morphology, size, and buoyant density. The latter two properties are shared by several groups of viruses; hence, the morphologic appearance of this human virus in stool specimens is critical to its current classification. The virus is 31 to 35nm in size and has characteristic cup-shaped (chalice) indentations observed by electron microscopy on the surface of negatively stained viral particles (174). Human calicivirus has not been cultivated in vitro, nor has it been shown to cross-react immunologically with animal caliciviruses.

Community and nosocomial outbreaks as well as sporadic cases of gastroenteritis associated with calicivirus have been reported from Japan, England, Canada, and Scandinavia (175-184). Some of the reported epidemiological and clinical features appear to be similar to those of rotavirus. Serum antibodies are rapidly acquired between 6 and 24 months of age and most older children and adults are seropositive (185). Symptomatic illness usually occurs in younger children but neonates seem to be spared. One gastroenteritis outbreak is described in a home for the elderly (179). Duration of calicivirus illness and symptoms are similar to those reported for rotavirus, but symptoms are described as milder with calicivirus (180).

Most laboratory studies with calicivirus have relied on electron microscopy and immune electron microscopy. Seroconversions and rises in calicivirus-specific IgM antibody have been demonstrated in ill persons by immune electron microscopy (176). In one study, calicivirus particles were not visualized in any of 7 stool specimens obtained by chance 2 to 10 days prior to a gastroenteritis outbreak in a Japanese orphanage (176). Subsequently, the virus was detected in 18 of 19 fecal samples collected within 4 days after the outbreak. These included samples of 3 asymptomatic infants who nonetheless

seroconverted.

Recently, a microtiter solid-phase radioimmunoassay has been developed for the detection of human calicivirus in stools (186). With the use of this technique, it is hoped that additional data with regard to the medical importance of calicivirus in human disease will be forthcoming.

As noted above, it has been stated that Norwalk virus may be a calicivirus based on its small size and polypeptide composition (123). However, no data are reported on any potential relatedness between human calicivirus and Norwalk virus. The reported sizes of these two viruses vary slightly with calicivirus described as 31 to 35 nm in diameter and Norwalk virus as 27nm. These measurements have been made in different laboratories, and careful comparative electron microscopic studies are needed. One additional difference between the two viruses is that antibody prevalence levels rise at a much earlier age for calicivirus than for Norwalk virus in populations residing in developed, nontropical areas.

Astrovirus

Small, round, virus-like particles, 29-30nm in size, have been observed by electron microscopy in the feces of infants with mild gastroenteritis and are named astroviruses based on their characteristic six pointed surface star morphology (187). Based on human volunteer studies (188), astrovirus seems to cause a transmissible infection of low pathogenicity for adults. An astrovirus-containing fecal filtrate derived from a child with mild gastroenteritis was given orally to 8 adult volunteers with the production of vomiting and diarrhea in one person who concurrently shed large amounts of virus. A fecal filtrate derived from this volunteer was then given

to 9 other subjects, none of whom developed vomiting or diarrhea and two of whom shed the virus.

Several pediatric gastroenteritis outbreaks have been associated with astrovirus (189-191). One Japanese outbreak affected 43 of 84 children in a kindergarten and astrovirus was visualized in 2 of 3 stool specimens examined by electron microscopy; all six paired sera obtained from ill children showed an antibody rise by immune electron microscopy (190). In one seroepidemiologic study in Great Britain, over 70 percent of children acquired serum antibodies to astrovirus by 3 to 4 years of age (192).

Astrovirus is identified in the laboratory primarily by electron microscopic techniques. It has not been propagated serially in vitro, although viral antigens are reported to appear in inoculated primary human embryonic kidney cells (188). Paired sera from individuals infected with Norwalk virus, calicivirus, and Otofuke agent (see Small Round Viruses below) failed to show a serologic response to astrovirus by immune electron microscopy (190).

Small Round Viruses

As a result of the extensive use of electron microscopy to examine human diarrheal stool specimens, a variety of noncultivatable small round virus-like particles have been observed. The first such particle recognized was the 27nm Norwalk virus (118) which is now a well established and important cause of human gastroenteritis. Other similar sized (25-35nm) virus-like particles have been observed, some of which lack a distinctive morphology (like Norwalk virus) and others which possess a distinctive and definable structure (like calicivirus and astrovirus).

Those small round viruses in the 25-35nm size range lacking a distinctive morphology have been referred to as Norwalk-like viruses or, in earlier literature, parvovirus-like viruses. These agents generally share properties of morphology, density and derivation from epidemics or family outbreaks of gastroenteritis. They are usually named by the geographic location of the outbreak or the presumed source of contagion. Some examples of Norwalk-like viruses are Hawaii, W-Ditchling, cockle, Snow Mountain, Marin Country, and Parramatta (193-198). By immune electron microscopy, the Norwalk,Hawaii, and W-Ditchling agents are serologically distinct, and the Marin County and Snow Mountain agents show antigenic differences from one another and from Hawaii agent and Norwalk virus. Thus, based on immune electron microscopy, there is some suggestion of 4 or 5 immunologically distinct agents; Norwalk, Hawaii, Marin County, Snow Mountain, and perhaps W-Ditchling. However, this evidence is based mostly on a relatively crude immunologic analysis using convalescent human sera, and definitive antigenic analysis is currently lacking. The infectious and pathogenic nature of Norwalk, Hawaii, W-Ditchling, and Snow Mountain agents has been shown in adult human volunteers, some of which have also demonstrated a lack of cross-protection between Norwalk and Hawaii agents in short term rechallenge studies (120). It is important to note that the medical importance of the Norwalk-like viruses, with the exception of Norwalk virus itself, remains to be established.

A somewhat larger (33 to 40nm) small round virus, the Otofuke agent, has been associated with several outbreaks of diarrhea among children and adults in Japan (199). By immune electron microscopy, the Otofuke agent is distinct from Norwalk virus, W-Ditchling and

calicivirus (200).

An overview of the small round viruses must conclude that although these agents are usually described within a single outbreak as being consistent in size and appearance, there is often variation among outbreaks. Some of this variation may be due to the interpretations of investigators, as well as to factors such as specimen storage, electron microscopic techniques including microscope calibration, and presence of copral antibody. Frequent exchange of materials among laboratories, development of immunoassays, and continued efforts to propagate the small round viruses are needed to help assess the role in human disease of these often observed but poorly understood agents.

CANDIDATE GASTROENTERITIS VIRUSES

Several virus-like particles demonstrable by electron microscopy in feces are putative gastroenteritis viruses but establishment is needed of their biological nature and significance. A good example is the coronavirus-like particles that have been observed in stool specimens from adults and children with gastroenteritis as well as from a similar proportion of healthy subjects in most studies (201). These fecal particles have been detected in various parts of the world (201-207). They are also reported to be excreted chronically by some individuals for months or years (202,206,208), a finding that further confounds attempts to associate coronavirus-like particles with disease. One report, requiring confirmation, noted these particles in the feces of a high proportion of hospitalized ill babies during an outbreak of severe necrotizing enterocolitis; the putative agent for this outbreak was propagated in cell culture, in contrast to the other reported coronavirus-like particles (204). However, no structural details of the original agent or immunologic

or biophysical characterization of the cultivated isolate have been published and the possibility of culture contamination by a coronavirus of bovine origin has been raised (201). In short, the presence of coronavirus-like particles in human stools has not been proven to be related etiologically with acute gastroenteritis and current characterization of these particles relies exclusively on morphological criteria.

There are also small round virus-like particles observed in stools that currently cannot be as clearly associated with gastroenteritis as are the Norwalk-like viruses and Otofuke agent described earlier. These include 33 to 40 nm particles termed minireovirus and minirotavirus that have been seen in some infants with diarrhea (183, 209,210).

FUTURE RESEARCH

Our current level of understanding of viral gastroenteritis is impressive when considered in the context that etiologic agents were first discovered a mere decade ago. It seems reasonable to anticipate substantial research progress in this field during the coming decade. Our general view is that laboratory research efforts should be concentrated at this time in three major directions. First, efforts at rotavirus vaccine development and clinical trials will almost certainly be accelerated. The current understanding of many features of this pathogen now justifies vigorous programs in this area. Second, the biology of Norwalk virus needs to be unraveled and techniques simplified to permit expanded epidemiologic surveillance and study of this virus by more laboratories. Finally, the epidemiological importance of certain gastroenteritis viruses should be determined; these viruses include enteric adenovirus, calicivirus, astrovirus, and Norwalk-like

viruses and other small round viruses. When this information is obtained, assessments can be made of the need for approaches at prevention and control of disease produced by these agents.

Table 1

Gastroenteritis Viruses

I. Produce Illness; Major Epidemiologic Importance Shown

A. Rotavirus

B. Norwalk Virus

II. Produce Illness; Major Epidemiologic Importance Not Shown

A. Enteric Adenovirus

B. Calicivirus

C. Astrovirus

D. Small Round Viruses

Examples: Hawaii, W-Ditchling,

Snow Mountain, Marin County, Otofuke

III. Suspected to Produce Illness: Epidemiologic Importance Not Shown

A. Enteric Coronavirus-Like Particles

B. Small Round Virus-Like Particles

Examples: Minireovirus, Minirotavirus

Table 2

Classification of Rotavirus

Type of Classification	Number of Types Designated	Viral Component Involved	Diagnostic Tests Used to Define Classification
Serotype	4	Antigenicity of outer capsid glycoprotein, the gene 8 or 9 product	Neutralization tests: plaque reduction, CPE neutralization, fluorescent focus reduction neutralization
Subgroup	2, possibly 3	Antigenicity of major inner capsid protein, the gene 6 product	Enzyme immunoassay, complement fixation, counterimmunoelectrophoresis, immune adherence hemagglutination
Electropherotype	Numerous	Migration pattern of genomic RNA in gel electrophoresis	Polyacrylamide gel electrophoresis

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